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The molecular basis of superficial scald development related to ethylene perception and α-farnesene metabolism in 'Wujiuxiang' pear

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ABSTRACT

Superficial scald is a major disorder that may occur in pear fruits following long-term cold storage. In this study, harvested 'Wujiuxiang' pears (*Pyrus communis* L.) were treated with 1.0 μL/L 1-methylcyclopropene (1-MCP), stored at 0 °C for 120 days, and transferred to 25 ± 2 °C for seven days to simulate shelf life. The effects of 1-MCP on scald development, the expression of ethylene perception genes, and α-farnesene metabolism and the expression of its associated genes were investigated. Compared with the control, 1-MCP reduced the scald index after 120 days of cold storage and during shelf life, suppressed the accumulation of α-farnesene and conjugated trienols (CTols), inhibited the expression of *PcETR2*, *PcERS1*, *PcHMGR2*, *PcAFS1*, *PcGPX5*, *PcGSTU7*, and *PcGSTU17*, and delayed the maximum expression of *PcETR1*, *PcERS2*, *PcHMGR1*, and *PcGPX6*. These results indicate that 1-MCP regulates the expression of target genes to inhibit ethylene action and α-farnesene metabolism, which contributes to slower scald development. These findings suggest that *PcGPX5*, *PcGPX6*, *PcGSTU7*, and *PcGSTU17* are involved in CTol accumulation and scald development in 'Wujiuxiang' pear fruit.

1. Introduction

The 'Wujiuxiang' pear (*Pyrus communis* L.) is favored for its excellent flavor quality and exhibits a high market value. However, this pear is highly susceptible to superficial scald, a physiological disorder that manifests as many irregular brown or black patches on the fruit peel, after long-term cold storage, which can rapidly expand at subsequent shelf life, resulting in severe fruit quality loss (Dong et al., 2012; Gao et al., 2015; Guan et al., 2014).

Ethylene plays a pivotal role in scald development in apples and pears. The internal ethylene concentration increases in climacteric fruit during storage and is related to scald development (Arquiza et al., 2005; Calvo et al., 2015; Whitaker et al., 2009). 1-Methylcyclopropene (1-MCP), an ethylene action inhibitor, significantly inhibits scald development in apples and pears (Bai et al., 2009; Busatto et al., 2014; Lu et al., 2013; Yazdani et al., 2011); 1-MCP inhibits ethylene production (Gao et al., 2015; Li and Wang 2009) and influences the expression levels of ethylene biosynthesis and receptor genes (Chiriboga et al., 2013a; Tsantili et al., 2007; Xie et al., 2014; Yang et al., 2013). However, ethylene biosynthesis and signal transduction are differently affected by 1-MCP in different species, cultivars, and storage phases (Chiriboga et al., 2013a; Tsantili et al., 2007). The mechanism of 1-MCP on scald inhibition in pear fruits and its relationship to ethylene responses needs further study.

It has been suggested that scald development is related to the synthesis and oxidation of α-farnesene, which accumulates at a relatively high level in the fruit peel during low-temperature storage (Calvo et al., 2015; Lu et al., 2013; Whitaker et al., 2009; Yazdani et al., 2011). The conjugated trienols (CTols) that result from the oxidation of α-farnesene are thought to play a causal role in the occurrence of scald (Giné Bordonaba et al., 2013; Whitaker 2007). It is generally accepted that the accumulation of both α-farnesene and CTols may be mediated by ethylene and effectively inhibited by 1-MCP treatment in some apples and pears varieties, which is correlated with scald development (Bai et al., 2009; Lu et al., 2013; Xie et al., 2014; Yazdani et al., 2011). However, recent findings have suggested that the a-farnesene accumulation and scald development may not be exclusively mediated by ethylene in some pear varieties (Calvo et al., 2015; Larrigaudière et al., 2016).

Abbreviations: 1-MCP, 1-methylcyclopropene; CTols, conjugated trienols; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; AFS, α-farnesene synthase; GPX, glutathione peroxidase; GST, glutathione *S*-transferase; CT, conjugated triene; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase.

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In apples and pears, three key groups of enzymes may be involved in α-farnesene metabolism: 1) 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), which initiates the cytosolic mevalonic acid pathway to synthesize sesquiterpenes (Rupasinghe et al., 2001); 2) α-farnesene synthase (AFS), which catalyzes the final rate-limiting step in α-farnesene biosynthesis (Gapper et al., 2006; Pechous and Whitaker 2004); 3) a currently hypothetical enzyme involved in the production of CTols, possibly glutathione peroxidase (GPX) or glutathione *S*-transferase (GST) (Whitaker 2012). It has been demonstrated that the transcription levels of *HMGR* and *AFS* genes are related to α-farnesene synthase and scald development, and may be inhibited by 1-MCP treatment (Gapper et al., 2006; Rupasinghe et al., 2001; Sabban-Amin et al., 2011; Tsantili et al., 2007). GPX or GST may reduce conjugated triene (CT) hydroperoxide to CTols based on the reducing activity of alkyl hydroperoxides to the corresponding alcohols (Dixon et al., 2010). Additionally, GPX and GST are certainly involved in fruit development and ripening (Huan et al., 2016; Shi et al., 2014), plant senescence (Chen et al., 2004; Kou et al., 2014), and abiotic stress (Chen et al., 2012; Dixon et al., 2010; Gill and Tuteja 2010). However, the roles of *GPX* and *GST* in CTol accumulation and scald development are unclear.

According to previous reports, ethylene action and α-farnesene metabolism are clearly involved in scald development in apples and pears. However, the precise mode of scald development has not been fully characterized to date, and the involvement of *GPX* and *GST* in scald development is unclear. Therefore, the objective of the current study was to understand the physiological and molecular basis of superficial scald development related to ethylene perception and α-farnesene metabolism, as well as to investigate mechanisms by which 1-MCP inhibits scald development in 'Wujiuxiang' pear.

2. Materials and methods

2.1. Materials and treatments

'Wujiuxiang' pear fruit were harvested in Jinzhou County (Hebei, China) at the commercial fruit maturity stage (August 29, 2012). Fruit were transported to the laboratory within 2 h. Uniform fruit (average weight: 289.0 g) without any visual defects were selected, and samples collected at day 0 were used as the initial samples for both control and 1-MCP-treated fruit. Thereafter, fruit were randomly divided into two lots. One lot was exposed to 1.0 μL/L 1-MCP (Rohm and Haas China Inc., Beijing) for 24 h at 25 ± 2 °C, and the other lot was exposed to air and used as the control. After treatment, each group was stored at 0 °C. The fruit were removed after 30 day intervals during cold storage for biochemical measurements. After 120 days of cold storage, the fruit were transferred to 25 ± 2 °C to simulate shelf life for seven days.

2.2. Scald index measurement

The scald data are expressed as a scald index based on the percentage of the fruit surface area affected (Zanella, 2003), where no scald = 0, <25% = 1, 25–50% = 2, and >50% = 3. The scald index was normalized to 100 by multiplying the values by 100/3. Scald incidence was determined as the percent rate of affected fruit. Three replicates were measured for each treatment, and 10 fruit were sampled in each replicate.

2.3. α-Farnesene and conjugated trienols content measurement

The extraction and analysis of α-farnesene and conjugated trienols were performed as described by Isidoro and Almeida (2006) with some modifications. Two discs (1 cm in diameter) were removed from one strip of peel taken from the equatorial portion of each pear. Twenty discs were collected and placed in tubes containing 20 mL of hexane for 2 h. Two milliliters of the extract was loaded on a Florisil column and eluted using 3 mL of hexane to measure absorbance at 232 nm. The absorbance of the remaining extract was measured at 281 and 290 nm. The contents of α-farnesene and the conjugated trienols per cm^2 of pear peel were calculated using the molar extinction coefficients ε_{232} = 27,740 for α-farnesene and $\varepsilon_{281-290}$ = 25,000 for the CTols. Three replicates were analyzed for each treatment with 10 fruit per replicate.

2.4. RNA isolation, DNA sequencing, and quantitative RT-PCR analysis

Total RNA was extracted using the EasySpin Plant RNA Extraction Reagent (Aidlab Biotechnologies Co., Ltd, Beijing, China) according to the manufacturer's instructions. All RNA extracts were treated with DNase I (TakaRa Biomedicals, Japan) and then purified following the manufacturer's instructions. Four replicates were analyzed for each treatment with 10 fruit per replicate.

First-strand cDNAs were synthesized from DNase-treated RNA (0.5 μg) using the TaKaRa RNA PCR Kit (AMV) Version 3.0 (TaKaRa Biomedicals). Quantitative RT-PCR was performed using the SYBR Premix Ex Taq™ (Perfect Real Time) Kit (TaKaRa Biomedicals) with the 7500 Real-Time PCR System (Applied Biosystems, USA). The PCR primers listed in Table 1 were designed using OMIGA 2.0. *PcActin2* served as an internal control. The qRT-PCR reaction was carried out in a final volume of 20 μL, which contained 10 μL of SYBR Green PCR Premix Ex Taq™, 0.4 μL of ROX II dye, 0.4 μL of forward and 0.4 μL of reverse primer, and cDNA equivalent to 10 ng of RNA. The reaction conditions were performed as follows: 10 s at 95 °C, 40 cycles of 95 °C for 5 s and 60 °C for 34 s. To confirm the specificity of amplification, the melting temperature of the amplification products was analyzed using a dissociation curve. All quantitative RT-PCR reactions were normalized using a Ct value corresponding to the *PcActin2* gene. The amplification efficiency of primers,

Table 1
Primers for quantitative RT-PCR analysis, the gene name, GenBank accession number, GDR accession number, forward and reverse primers, and product length are shown.

Gene	GenBank Accession NO.	GDR Accession NO.	Forward (5′-3′)	Reverse (5′-3′)	Product (bp)
PcETR1	AF386509	PCP024250	AGTCTAAGCAGCCTTTTGCACC	TGCTGACCCCATTATCATCC	99
PcETR2	HM561909	PCP002455	GATCCTGGAGAATCATCAGAGC	GCAGTTACAATGCAACCAAGC	137
PcERS1	AF386515	PCP004450	CGTTGCATTCATCGTCAAACTG	TCGATGAACTTGCGCCAAATCG	158
PcERS2		PCP026971	CGTTGCATTCATCGTCCAACTA	TTCGACGAAGTTACTCCGAAAC	159
PcHMGR1	KF861867	PCP023794	GTTCTCACTGCATTACCATG	TCAGACAAGCGGATTGAGATG	132
PcHMGR2		PCP017787	CAGTTGGAGGAGGAACCCAG	AGTTTGAACCGGGTGAGTC	93
PcAFS1	DQ309034	PCP028486	AATGGTTGGAACCAAGTATTACC	GAAACTGATGATGAATCGCATCC	149
PcGSTU7		PCP024796	ATGGTTCCGGTGTTTGTCCA	GAGGGTCATTTGGCAGCAGA	109
PcGSTU17		PCP036861	GGTCAAACTTCTGGACGACG	ATCTTAGCAAACTCAGCCAGC	123
PcGPX5		PCP038893	GGGACAAGTCAGGATGCTGAAC	ACAGGTTCTGTATCCGGCCCA	101
PcGPX6		PCP035932	AGACCCTTTTGCCACGGTCG	CGGACTGGCCAGCCATTGTA	114
PcActin	AB190176	PCP017023	GCTGAGAGATTCCGGTGCCC	TTGACCCACCACTGAGCACG	157